Reversible mechanosensitive ion pumping as a part of mechanoelectrical transduction

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ABSTRACT To explain the ability of some mechanosensitive cells to reverse the process of mechanotransduction and to generate mechanical oscillations and emit sound, a piezo-conformational coupling model (PCC model) is proposed. The model includes a transport protein which changes either its volume (PV-coupling) or its area in the membrane (γA-coupling) when undergoing conformational transitions. Such a protein can interact with an oscillating pressure to pump ions and create a transmembrane gradient if the affinities of the protein for ions are different at the two sides of membrane. The frequency and concentration windows for mechanical energy transduction were determined. Under optimal conditions, the efficiency of energy transduction can approach the theoretical maximum of 100%. If the concentration gradient exceeds the static head value (quasi-equilibrium which can be built up and maintained by this transport system), the energy transduction reverses and the transporter becomes a generator of mechanical oscillations at the expense of a concentration gradient.

Estimation of thermodynamic parameters of the pump shows that the PV-coupling model would require large pressure oscillations to work while the γA-coupling model could work in physiological conditions. The γA-coupling mechanism may be used by cells for two purposes. In the reverse mode, it can be a force generator for various applications. In the direct mode, it may serve bioenergetic purposes by harvesting the energy of mechanical oscillations and storing it in the form of a concentration gradient. This pump has an unusual thermodynamic feature: it can distinguish the two components of the electrochemical potential gradient, i.e., the concentration gradient and the electrical potential, the latter serving as a permissive switch to open, or close, the pump when the potential reaches the threshold value.

Predictions of the PCC model and its probable involvement in biological mechanotransduction are dicussed.

INTRODUCTION

In the preceding investigations, an electroconformational coupling (ECC) model for membrane transport was developed which could transduce the energy of an oscillating electrical field into the concentration gradient of a ligand (1–12). It was claimed that oscillation of other parameters coupled to conformation transition of a transport protein can also generate the active transport and energy transduction (12, 13). This principle is applied here to explain some properties of the mechanosensitivity (14–22) and other pressure sensitive membrane transport activities.

Mechanoelectrical transduction is usually understood as a one way transduction of mechanical signal by controlled release of energy stored in an ion gradient. This type of mechanosensitive (MS) channel is a passive transducer which uses the mechanical signal as a trigger to initiate an ion flux down the electrochemical potential gradient.

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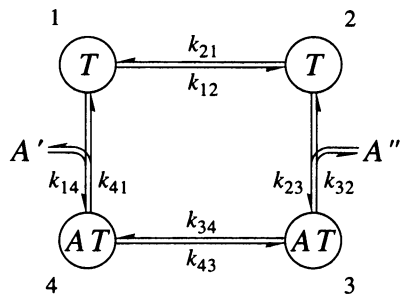
However, there are some observations which do not fit into this conventional picture of mechanotransduction. The cochlea can receive acoustic signals and tune sharply to certain frequencies (23, 24). It can also emit sounds either spontaneously (25) or in response to acoustic stimuli (26, 27). Until now, no satisfactory explanations have been given as to how hair cells in the cochlea can absorb, temporarily store, and re-emit energy (23).

To describe this active part of mechanosensitivity phenomenon, we suggest a piezo-conformational coupling (PCC) mechanism which can interact with an oscillating pressure, or acoustic wave, to harvest mechanical energy, and actively transport ions up its concentration gradient, thus, converting acoustic energy into an electrochemical gradient. When reversed, this mechanism can convert the electrochemical gradient back into mechanical oscillations which could simulate the process of sound emission. The PCC model is not intended to substitute our current understanding of the mechanoelectric transduction mechanism. The PCC model reflects only an active part of the reversible mechanotransduction in the membranes. Therefore, it can be a part of a more general picture of the mechanosensitivity.

PIEZO-CONFORMATIONAL COUPLING AND TRANSPORT

Consider a transmembrane protein that can transport a molecule or an ion, A, between two neighboring solutions. Let this protein have two distinct conformational states, T_1 with the binding site for A exposed to the left side, and T_2 with the binding site for A exposed to the right side. Suppose that during these conformational changes some geometrical parameter of the protein is also changing such that conformational transitions can be influenced by the varying pressure. For a protein in solution this parameter can only be the volume of the molecule (Fig. 1 *a*). For membrane proteins there is another option, membrane tension. The parameter conjugated to membrane tension is the area occupied by this protein in the membrane. If this area is changing with protein conformation then these transitions can be influenced by the membrane tension (Fig. 1 *b*). In this case, change of area in the membrane can occur without change of the protein volume. Therefore we shall distinguish between two types of piezo-conformational coupling, the pressure-controlled coupling (PV-coupling) and the stretch-controlled coupling (γA-coupling). A simple cycle for the transporter can be described (28) by the four-state kinetic model:



(1)

When changing its conformation from left to right, the protein molecule changes its volume by ΔV or area in membrane by Δa, which can be referred to as a gating volume and area, respectively. Let $z_T e_o$ be a gating charge, $z_A e_o$, charge of transported particle, φ, transmembrane potential in Volt, $\Psi = F\varphi/RT$, a dimensionless membrane potential, and $\rho = P\Delta V/RT$, the dimensionless pressure. The conformational transitions of both the loaded and the unloaded transporter must occur in the interval between the minimal and maximal pressures ρ_{min} and ρ_{max}. To accomplish this, the extreme pressure values should satisfy the double inequality

$$\rho_{min} \ll z_T\Psi + \ln K_{21}, (z_A + z_T)\Psi + \ln K_{34} \ll \rho_{max}, \quad (2)$$

where $K_{21} = k_{12}/k_{21}$ and $K_{34} = k_{43}/k_{34}$.

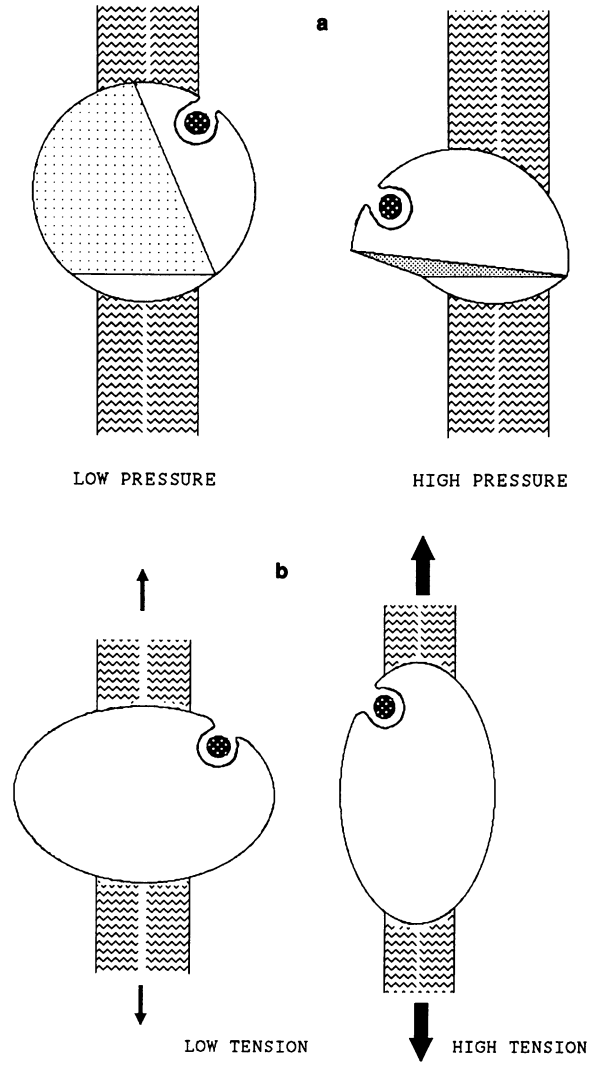


FIGURE 1 Two orientations of membrane transporter coupled to (*a*) pressure (PV-coupling) and to (*b*) membrane tension (γA-coupling). At low pressure (tension) the binding site of a protein is oriented to the right and at high pressure (tension) to the left. The transporter is presented in the form loaded with a particle *A*.

MEMBRANE FLUX

The method of solving kinetic equations of Scheme 1 for the square-wave oscillations of pressure, was described in reference 12. If applied here, it gives the following results.

Frequency window

In the limit of large pressure oscillation, membrane flux *J* as a function of the oscillation frequency *f* is shown in Fig. 2 by the curve with a broad maximum, or plateau.

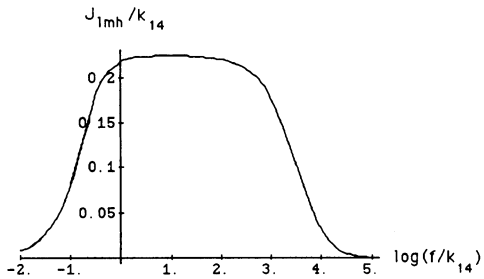


FIGURE 2 Frequency dependence of flux for the antisymmetric transporter. The expression for the flux (J_{1mh}) is defined in reference 28. Equilibrium constant $K_{14} = 0.1$, concentrations $A' = A'' = 1$ and $\beta = 100{,}000$ were used in the calculation.

The magnitude of a flux at this plateau is

$$J = \frac{1}{2}\frac{(K_{41}A' - K_{32}A'')}{(1 + K_{41}A')/k_{32} + (1 + K_{32}A'')/k_{41}}. \quad (3)$$

The left border of this optimum window is

$$f_{left} = \frac{k_{14}(K_{14} + A)(1 + K_{14}A)}{2(1 + A)(1 + K_{14})} \quad (4)$$

and the right border is

$$f_{right} = \frac{\beta k_{14}}{4}\sqrt{\frac{K_{14}}{3A}}. \quad (5)$$

For simplicity, in Eqs. 4 and 5 we adopted the condition of antisymmetry of transporter (12) and took $A' = A'' = A$. Parameter β is the ratio between the rates of conformational transitions and chemical reaction, $\beta = k_{12}\exp[(z_T\Psi\text{-}\rho_{min})/2]/k_{14}$. The position of a maximum on the frequency axis is

$$f_{max} = \sqrt{f_{left}f_{right}}. \quad (6)$$

Concentration window

The concentration dependence of the flux, when $A'' = A' = A$, is also described by the curve with a plateau similar to Fig. 2. Therefore, this phenomenon displays frequency and concentration windows. The dependence of pump flux (Eq. 3) on one-side concentration A'', while the other concentration A' is kept constant, is presented in Fig. 3 *A*. With increasing A'', the flux from left to right decreases and eventually reaches zero. The transmembrane concentration difference at that static head is given by the same equation $A''_{sh} = A'K_{23}/K_{14}$ found for the ECC model (12).

If A'' is increased beyond the static head value, the flux changes its sign and can drive the pump in the opposite direction, transforming the pump into a generator of mechanical oscillations.

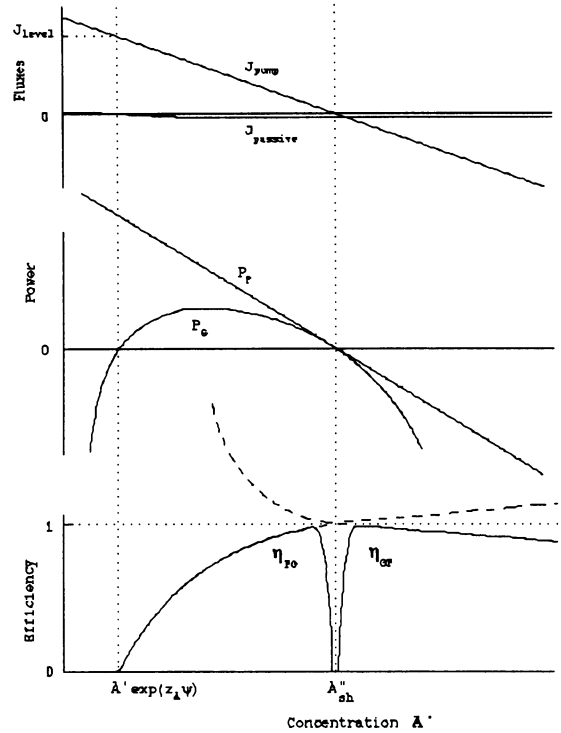


FIGURE 3 Transport flux J, power P_P supplied by an oscillating pressure, power P_G applied to the gradient of electrochemical potential, efficiency η_{PG} of energy transduction from the oscillating pressure to the gradient of electrochemical potential, and efficiency η_{GP} of energy transduction from the concentration gradient to the mechanical oscillations, all vs. concentration A'' for fixed concentration A'. The curves are drawn from Eqs. 3, 8, 9, 10 with $k_{41} = k_{32} = 1$, $K_{32} = 0.1$, $K_{41} = 0.5$, $A'' = 2$, $\psi = 0$.

Roles of two components of electrochemical potential

It is interesting that the static head depends only on the ratio of the concentrations of A, but not on the membrane potential, even if particle A is an ion. This might seem unusual because it is widely accepted in bioenergetics that the two components of an electrochemical potential gradient should manifest together, at least when the system is close to equilibrium (e.g., near the static head). Close to this point a pump should work against the difference of electrochemical potentials of a given species rather than against each component separately. But this happens not to be the case for the PCC pump.

Then what is the role of membrane potential in this mechanism? The plot of active flux J as a function of

membrane potential (Fig. 4) is close to a step function, dropping from some constant value to zero when electrical potential reaches the threshold. The position of this threshold depends on the charge numbers z_A and z_T. If $z_A < 0$ and $z_T = -z_A$ then

$$\Psi_{th} = (\rho - \ln K_{12})/z_A. \quad (7)$$

Therefore, the membrane potential plays the role of a permissive switch which opens and locks the pump. It works both for charged and neutral particles. The combined dependence of pump flux on two components of the membrane gradient, concentration and electric potential, is presented in the three-dimensional plot of Fig. 5. One can see that when the electric potential reaches the threshold, the curve flattens and the pump current approaches zero.

Eq. 7 includes the amplitude of pressure oscillations ρ. Therefore, if the pressure oscillations are large enough to cause this mechanism to start pumping, then the response, membrane current, will have the standard form which is independent of pressure amplitude. In that sense the piezo-conformational mechanism reacts to the external stimulus by the "all or none" principle and the threshold of the response can easily be shifted by a change in membrane potential.

EFFICIENCY OF ENERGY TRANSDUCTION

As in reference 12, we find that the power drawn by the transporter from the oscillating pressure is

$$P_p = J[\ln(K_{23}/K_{14}) - z_A\Psi], \quad (8)$$

and the power exerted by the transporter on the gradient of electrochemical potential is

$$P_G = J[\ln (A''/A') - z_A\Psi]. \quad (9)$$

The efficiency of the energy transduction is

$$\eta_{PG} = \frac{P_G}{P_p} = \frac{\ln (A''/A') - z_A\Psi}{\ln (K_{23}/K_{14}) - z_A\Psi}. \quad (10)$$

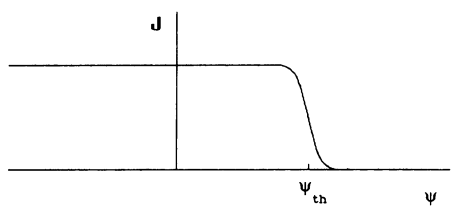


FIGURE 4 Dependence of active flux on membrane potential.

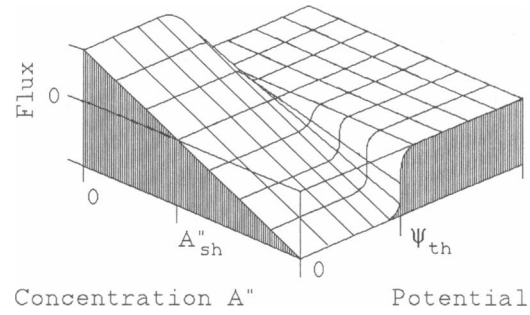


FIGURE 5 The three-dimensional plot of pump flux as a function of two components of membrane potential, concentration and electric potential.

The efficiency (Eq. 10) is presented in Fig. 3 as a function of A''. The highest efficiency is close to 100% and occurs near the static head.

If $A'' > A'K_{23}/K_{14}$ the energy is transduced in the opposite direction, i.e., from the concentration gradient to mechanical oscillations. In this case the efficiency of the reciprocal transduction may be defined as $\eta_{GP} = P_P/P_G = 1/\eta_{PG}$.

GATING COMPLIANCE

When the membrane is strained by external tension γ, the variation of the membrane area is partially determined by its elasticity and partially by conformational transitions of the transporter (29). The total compliance, $1/\kappa$, is the sum of bilayer compliance $1/\kappa_b$ and the compliance due to conformational transitions of the transporter, $1/\kappa_t$:

$$\frac{1}{\kappa} = \frac{1}{\kappa_b} + \frac{1}{\kappa_t}. \quad (11)$$

The reciprocal quantity κ is stiffness. The transporter compliance can be found as

$$\frac{1}{\kappa_t} = n\Delta a \frac{dP_{left}}{d\gamma}, \quad (12)$$

where n is the surface density of the transporters, and P_{left} is the steady-state probability of the left conformation of the transporter. This probability is equal to the sum of populations T_1 and AT_4:

$$P_{left} = T_1 + AT_4 = \frac{(K_{12} + A)(1 + e^{\xi})}{(K_{12} + A)e^{\xi} + (1 + K_{12})(1 + A) + (1 + K_{12}A)e^{-\xi}}, \quad (13)$$

where for simplicity we used the condition of antisymme-

try of the transporter (12), choosing equal concentrations $A' = A'' = A$ and set $z_T = 0$. The exponent ξ is

$$\xi = b + \frac{\gamma\Delta a - z_T e_0 \phi}{kT}, \tag{14}$$

where b is a constant and kT retains its usual meaning (Boltzmann's constant times temperature). Then

$$\frac{1}{\kappa} = \frac{1}{\kappa_b} + \frac{n(\Delta a)^2(K_{12}+A)\,(1+K_{12}A)\,(e^{\xi}+2+e^{-\xi})}{kT[(K_{12}+A)e^{\xi}+(1+K_{12})\,(1+A)\,+(1+K_{12}A)e^{-\xi}]^2}. \tag{15}$$

The plot of κ is presented in Fig. 6. One can see that the stiffness decreases in some regions where the transporter undergoes conformational transition. The position of the minimum on the axis of tension depends on membrane potential in a linear manner. It also depends on the solute concentration, which can be used for experimental verification of the theory.

DISCUSSION

Comparison of the PV-coupling and the γA-coupling mechanisms

Let us begin with the pressure-controlled (PV-coupling) model and take a reasonable estimate of pressure oscillations ΔP of 1 atm. To cause a tangible effect, this pressure must exert on the transporter a work of $W = 1$ kT. Then the volume change of a protein should be

$$\Delta V = W/\Delta P = 1\,kT/1\text{ atm} = 40\text{ nm}^3. \tag{16}$$

Zimmerberg and Parsegian (30) have estimated the internal volume change of transport protein during the opening and closing of an ionic channel from rat liver and from Neurospora, reconstituted into planar lipid bilayers. Applying osmotic stress, they found a change of 20–40 nm^3! These numbers would nicely fit the value estimated by Eq. 16, but unfortunately the experiments with osmotic stress do not characterize the volume change of the protein itself, which could be found only from the hydrostatic pressure dependence of the channel opening probability.

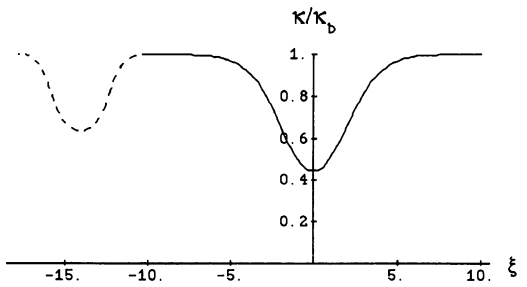


FIGURE 6 Stiffness of a membrane with PCC transporters as a function of dimensionless membrane tension. Curve is plotted according to Eq. 15 with parameters $A = 1$, $K_{12} = 0.1$, $\kappa_b n(\Delta a)^2/(kT) = 5$. The dashed part of the curve presents the superposition of the effect of another mechanism of compliance.

In the experiments with the hydrostatic pressure, Conti et al. (31) found that opening a sodium channel of Squid giant axon involves a net volume increase of ~26 Å^3 and an activation volume of ~58 Å^3. Clearly, these values are three orders of magnitude smaller than what is necessary to make the pressure-controlled PCC model workable. Hence the PV-coupling model may not be adequate for a biological system in physiological conditions. Only in vitro, one could try to create the conditions necessary for this model. We will return to this point later.

Now consider a stretch-controlled coupling model. The lipid membrane can endure the maximum tension of γ = 6–8 mN/m (32, 33). To produce a work $W = 1\,kT$ with tension γ = 6 mN/m, a membrane molecule must change its area by

$$\Delta a = W/\gamma = 0.7\text{ nm}^2. \tag{17}$$

This is roughly the area occupied by one lipid molecule in the bilayer. Membrane proteins usually occupy a much larger area and in the process of conformational change, such a variation of area can be easily anticipated. More importantly, this area change does not imply the concomitant volume change of the protein. It can be easily achieved either by the opening of the channel or by rotation of the protein in the membrane (Fig. 1 *b*).

If one assumes that area change of the protein transformation reaches 3 nm^2, then the stretch-controlled PCC mechanism has the capacity to generate membrane potential equal to

$$\varphi = \gamma\Delta a/e_0 \approx 100\text{ mV}. \tag{18}$$

Such membrane potential is of the order of magnitude which could be easily used in bioenergetics and, hence, the γA-coupling model meets this criterion.

The estimates made above are rather close to the elasto-geometrical parameters of mechanosensitive cells. The variation of area in the process of channel opening can be estimated for mechanosensitive channels of *Escherichia coli* (20). This channel has Δa = 1.65 nm^2. If the data of Zimmerberg and Parsegian (30), quoted above, are recalculated for the area variation, they yield Δa in the range from 3.5 to 7 nm^2. This is an even higher value than the cautious estimate of Eq. 17. Therefore,

the stretch-controlled PCC model could easily function in a biological system.

Variation of current and potential with time

When acoustic oscillations activate the PCC model, the ions flow through a membrane, changing its potential. Hence the system can shift to the other state where the amplitude of the pressure oscillations is no longer sufficient (Eq. 2) to maintain membrane current, and pumping will stop.

To estimate the characteristic time of this process, let us consider a cell with the radius $R = 1$ μm, specific membrane capacitance $C = 1$ μF/cm^2, and the number of transporter molecules $n = 1{,}000$. Let this transporter pump univalent ions under the influence of an acoustic signal with frequency $f = 10$ kHz. The change of potential $\Delta\varphi = 100$ mV can be reached in time

$$t = 4\Delta\varphi\pi R^2 C/nfe_0 = 10 \text{ ms}. \quad (19)$$

This performance is reasonable, but probably not adequate for the fastest signal receptions found in MS cells. Still it could be used in cases which do not demand very high speeds of reaction and it definitely can be used for bioenergetic purposes and for reversion of mechanotransduction and generation of mechanical oscillations.

Where the PCC mechanism may be involved and how it can be detected

The PCC model could explain a number of phenomena related to mechanotransduction (23). Some MS cells display rather sharp frequency selectivity. How hair cells become tuned to their appropriate frequencies remains unknown, though in a recent review, A. J. Hudspeth emphasized that "there is a possibility that tuning is sharpened by force-producing elements" (23). The PCC mechanism conforms with this concept, because it has a frequency window. As was shown above, this model predicts an optimum frequency. Working in parallel with the main MS channels, the PCC mechanism could contribute to the frequency tuning of the cell.

The contribution of the PCC mechanism should depend on the concentration of transported ions because, along with the frequency window, there is concentration window. Therefore, the PCC model predicts that the frequency tuning should depend on the concentration of ions, and this dependence should have a maximum at some intermediate concentration.

The reversibility of the PCC mechanism and its ability to generate oscillation may explain the sound emission by cochlea and some cells in birds and amphibians (23). To emit sound, the transporters in the PCC mechanism should move synchronously. The simplest way to synchronize them is to use enforced oscillations (10). This means that the generation of mechanical oscillations starts in response to external oscillations and continues on its own if there are resonant structures in the system. The frequency window should manifest itself as well, demonstrating a limited band of frequencies which may be generated and amplified by this mechanism. This is a crucial prediction of the PCC model which could be checked with experiments.

Another prediction of the model is that generation of mechanical oscillations depends on the initial concentration difference between the cytoplasm and the external medium of the cell. Mechanical oscillations would be generated only if this gradient is greater than the static head. A crucial test of the PCC model would be to detect mechanical oscillations when the cytoplasmic and external ligand concentrations are similar. Any mechanical oscillations under such conditions would rule out the PCC mechanism as a cause of these oscillations.

It was observed (34, 35) that an isolated hair cell under electrical stimulation shortens when depolarized and elongates when hyperpolarized (23). This observation is easily explained by the PCC model. If the transport protein is voltage sensitive, then membrane potential could cause the conformational transition and result in an increase in the area of cell membrane. Such a transition could manifest itself in a transient change of the cell capacitance due to a shift of the gating charge and an increase of the membrane area. Therefore, it would be interesting to detect this component of capacitive current. The PCC model can explain also the observation that the bundles of electrically resonant hair cells display back and forth motion in phase with the electrical signals (23).

The hair bundle demonstrates 40% more flexibility near its resting position than at the extremes of positive or negative displacement (29). This augmented compliance was associated with the gating of transduction channels. As was shown above, the PCC model also displays a drop of stiffness when the membrane tension causes the conformational transition of the transport protein. If the passive MS channels and PCC mechanism work together, then this curve should display two wells, if they are not superimposed (Fig. 6). Howard and Hudspeth (29) in their experiments with hair cells, found a central well near the resting position of the hair bundle, and in addition they observed the drop of bundle stiffness at the extreme of negative displacement. This drop is consistently observed in all the plots in their paper. It could be a manifestation of PCC mechanism. It would be interesting to check other predictions of this model. For example, the position of PCC minimum

should be both potential and concentration dependent. This dependence would be a crucial check for the PCC model.

If two stiffness wells due to the MS channel and PCC mechanism overlap, then one could resolve them using the voltage and concentration dependencies of the position of the PCC minimum, Eq. 15. Of course, the position of the MS channel well could also be voltage or concentration dependent, but it is unlikely that these dependencies are the same. Therefore, in principle these two minimums could be split under certain conditions, which would be another prove of the PCC model.

Other kinetic manifestations of pressure

Besides mechanosensitivity effect per se, pressure can cause other phenomena. Recently, G. Fortes and E. Almendares reported the paradoxical effects of hydrostatic pressure on (Na,K)-ATPase from dog kidney (38). Pressure in the range of 1–800 bar inhibited ATPase activity in the presence of both Na and K. However, without *K*, increasing pressure stimulated the ATPase activity up to 300% at 400 bar and the effect depended on sodium concentration. The stimulation was larger at lower Na concentration (400–500% at 400–800 bar with 25 mM Na). At higher Na concentration (0.4 M Na), the stimulation was smaller: 20–50% at 200–400 bar, which was followed by inhibition at higher pressure. The authors have suggested that intermediates of the ATPase reaction that contain occluded cations are favored by high pressure because they occupy a smaller volume. In the presence of Na and K, deocclusion of K would be inhibited by pressure thus inhibiting turnover. In the presence of Na alone, high pressure would favor Na binding and stabilize EP(Na_2), which hydrolyzes ATP faster than EP does, and, at higher Na, high pressure would stabilize EP(Na_3) which has a lower turnover rate.

The effect of isotropic hydrostatic pressure may be explained by the PV-coupling model. An estimate according to the PCC model gives a pressure required to induce these observed effects to be of the order of 1,000 atm. This is exactly the range of pressure which was exploited in the experiments by Fortes and Almendares (38). In addition they reported the bigger activation volume of ATPase than that by Conti et al. (31) for ion channel. Therefore, the PV-coupling version of PCC mechanism can work in this case.

Up to now, only constant pressure was investigated in relation to ATPase activity. The PCC model predicts that hydrolysis should be stimulated by oscillating pressure. It would be also interesting to find out if the active transport could be generated in these conditions by ATPase in the absence of other sources of energy.

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